# Monitoring the progression of breast cancer in an orthotopic-4T1 mouse model using single transducer harmonic motion imaging

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## Introduction

- Breast cancer is the second leading cause of death from cancer in women in the USA[1].
- Mammography and ultrasonography are used as screening tests for breast cancer[4].
- Collagen density is associated with increased mammographic density[2].
- Study found that stiffness of breast cancer tumor in mouse model was correlated to the amount of collagen[3].
- Ultrasound elastography (USE) is a non-invasive technique that assesses the elasticity of tissues by measuring tissue displacement when a force is applied[4].
- Acoustic radiation force (ARF) uses sound waves to displace tissue[5].
- Harmonic motion imaging (HMI) is an ARF-based method that uses a focused ultrasound transducer to generate harmonic oscillation and an imaging transducer to track the harmonic oscillation[6].
- Instead of two different transducers, single transducer harmonic motion imaging (ST-HMI) uses single ultrasound transducer to generate and track harmonic oscillation to facilitate data acquisitions[7].
- ST-HMI at single frequency indicated that the stiffness of a tumor increased over time relative to non-cancerous background tissue in a breast cancer mouse model[6].
- Instead of acquiring data at one frequency data at a time, ST-HMI was extended to collect 100-1000 Hz oscillation data in a single acquisition. Thus, the method is called multi-frequency ST-HMI[7].

## Objective

- Monitor the progression of tumor in an orthotopic-4T1 mouse model using multi-frequency ST-HMI and investigate the performance of each oscillation frequency

## Methods

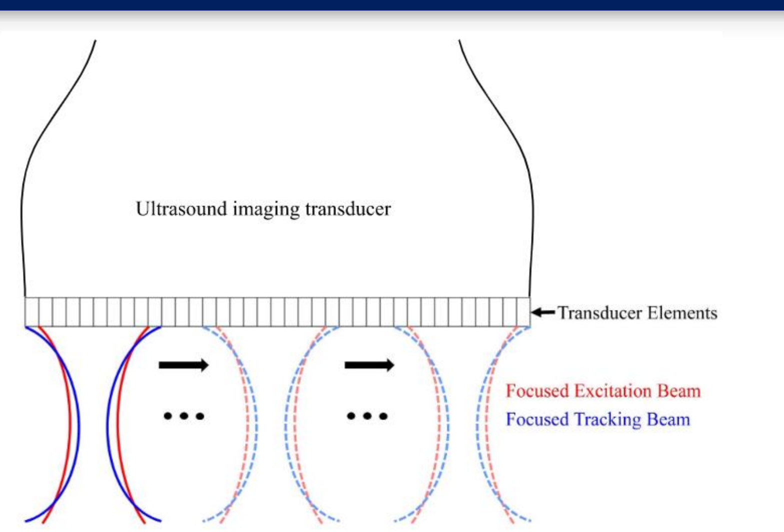


**Figure 1.** Pulse sequence of focused push and tracking pulses move electronically across lateral field to generate 2-D image[6].

- Multi-frequency excitation (push) pulse is a sum of sinusoids with frequencies of 100-1000 Hz and increments of 100 Hz (Fig. 2, Equ. (1)).
- Continuous multi-frequency push pulse was sampled to generate discrete push pulses (Fig. 2).
- Tracking pulses were transmitted in between the discrete push pulses to estimate the motion in the tissue (Fig. 2).
- Displacement versus time profile were generated for each pixel (Fig. 3b).
- Differential displacement (DD) was calculated for each pixel (Fig. 3c).
- Fourier Transform of DD was used to determine values of bandpass filter for the oscillation frequencies (Fig. 3d).
- DD was filtered at each oscillation frequency and pixel to generate P2PD image (Fig. 3e)[7].

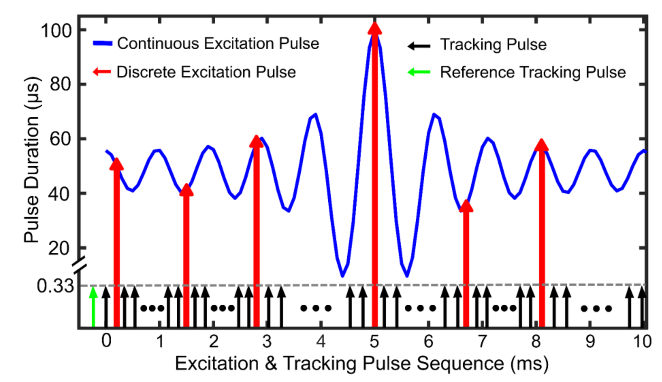


**Figure 2.** One cycle of multi-frequency ST-HMI pulse sequence with the continuous push pulse (blue) and the tracking pulses (black) in between the discrete push pulses (red)[7].

$$e_1(t) = \sum_{j=1}^{N_{sinusoid}} j^2 \times \cos(2\pi f_L t + \theta_j)$$

$$where\ \theta_j = \begin{cases} \pi, if\ j\ odd \\ 0\ if\ j\ even \end{cases}$$

**Equation 1.** Equation for multi-frequency excitation pulse as a sum of sinusoids. $N_{sinusoid}$ is an integer that represents the total number of sinusoids, and $f_L$ is the lowest frequency. $N_{sinusoid}$ = 10. $f_L$ = 100 Hz[7].

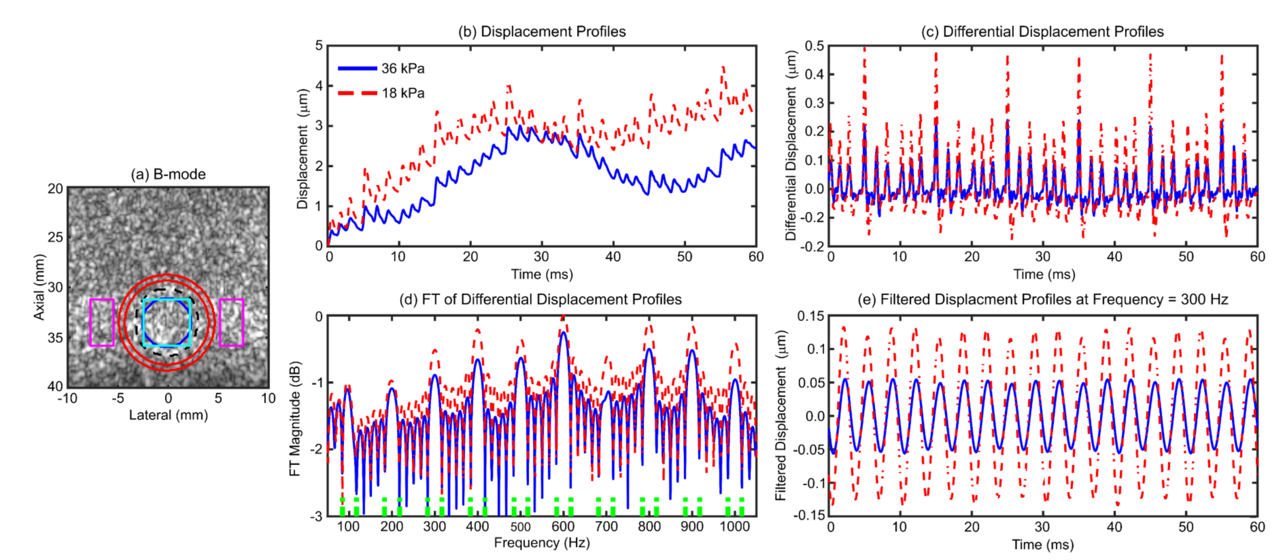


**Figure 3.** (a) B-mode ultrasound image of 6.5 mm phantom inclusion with Young's modulus of 36k Pa. Young's modulus of background was 18 kPa[7]. Black dashed line represents inclusion boundary. Circles and rectangles represent regions of interest in inclusion and background. ST-HMI derived (b) displacement profiles (c) differential displacement between successive time points (d) magnitude spectrum of Fourier transform (FT) of the differential displacement profiles (e) filtered displacement profiles at 300 Hz in inclusion (blue) and background (red). Green dashed line represents values of bandpass filter[7].

## Experimental Setup

- 8-10 week old female BALB/c mice were anesthetized (1- 2% isoflurane in oxygen) and placed in supine position on heating pad.
- Mice were injected with $2 \times 10^5$ 4T1 breast cancer cells in the 4th inguinal mammary fat pad[6].
- ST-HMI was performed using Verasonics Vantage Ultrasound System (Vantage 256, Verasonics Inc., Kirkland, WA, USA) with an L11-5 linear array transducer[6,7].
- ST-HMI-derived peak-to-peak displacement (P2PD) images were generated at oscillation frequencies of 100-1000 Hz.
- Mice were imaged 7, 12, 18, and 25 days after tumor injection[6,7].

## Results

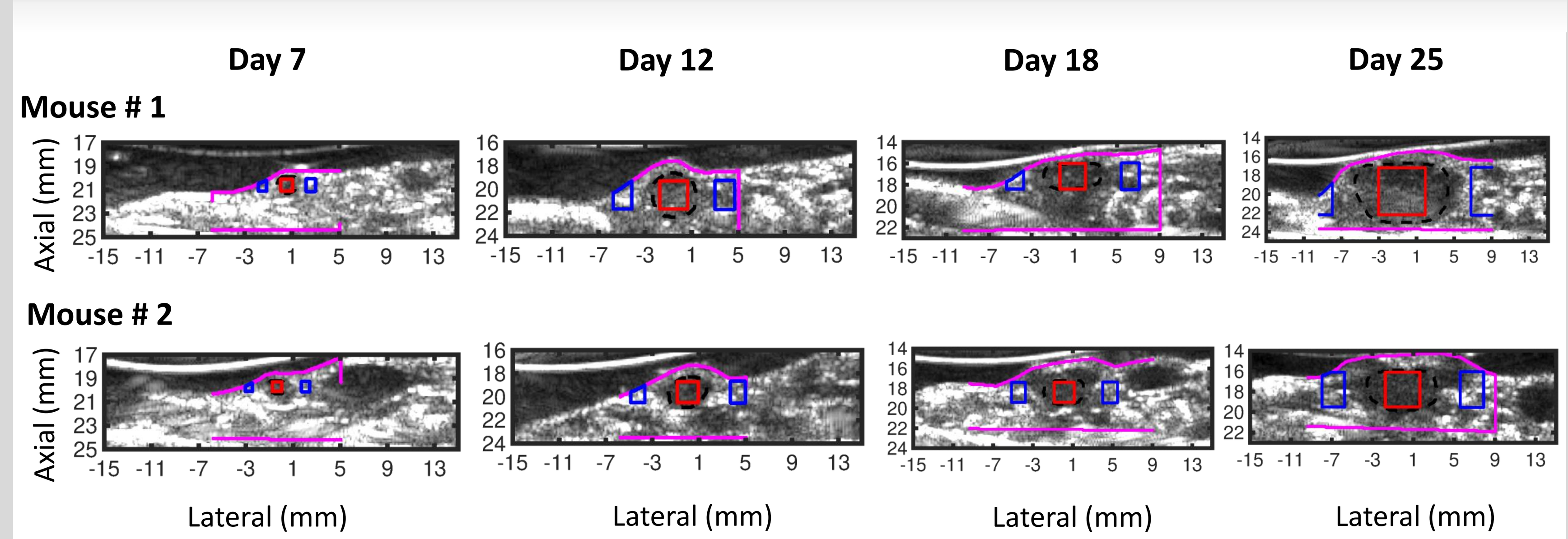


**Figure 4.** B-mode ultrasound images of mouse # 1 and # 2 tumors on Day 7 (first column), Day 12 (second column), Day 18 (third column), and Day 25 (fourth column). Magenta, black, red, and blue contours represent the field of view for the displacement images, the boundary of the tumor, the region of interest, and the background tissue, respectively.

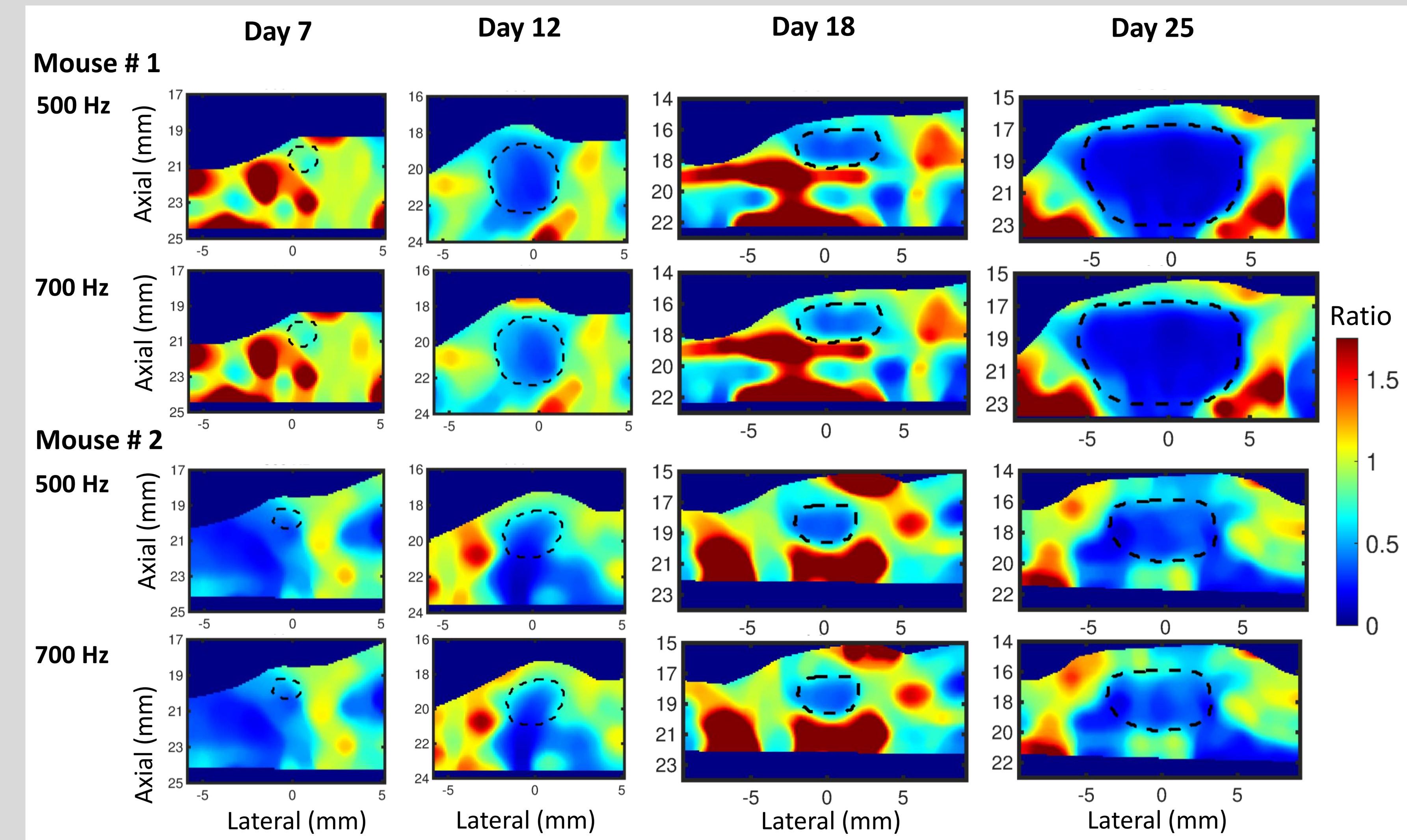


**Figure 5.** ST-HMI derived normalized peak-to-peak displacement image at 500 and 700 Hz of mouse # 1 and # 2 tumors on Day 7 (first column), Day 12 (second column), Day 18 (third column), and Day 25 (fourth column).

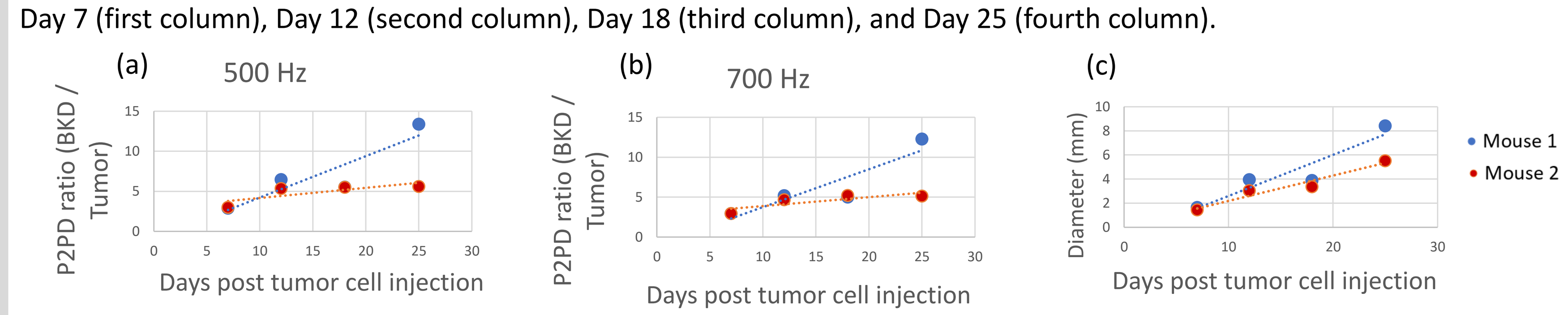


**Figure 6.** P2PD ratio at 500 Hz (a) and 700 Hz (b) versus time after tumor cell injection for Mouse # 1 (blue) and Mouse # 2 (red). (c) Tumor's diameter of Mouse # 1 (blue) and Mouse # 2 (red) versus time after tumor cell injection.

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|---|---|---|---|---|---|---|---|---|---|---|
| **Mouse #1** | | | | | | | | | | |
| Oscillation Frequency (Hz) | 100 | 200 | 300 | 400 | 500 | 600 | 700 | 800 | 900 | 1000 |
| Slope: P2PD versus time | 0.33 | 0.61 | 0.69 | 0.6 | 0.52 | 0.49 | 0.47 | 0.46 | 0.44 | 0.44 |
| $R^2$ : P2PD versus time | 0.8 | 0.8 | 0.84 | 0.88 | 0.81 | 0.83 | 0.82 | 0.82 | 0.83 | 0.84 |
| **Mouse #2** | | | | | | | | | | |
| Oscillation Frequency (Hz) | 100 | 200 | 300 | 400 | 500 | 600 | 700 | 800 | 900 | 1000 |
| Slope: P2PD versus time | -0.045 | 0.069 | 0.034 | 0.046 | 0.13 | 0.12 | 0.11 | 0.1 | 0.1 | 0.06 |
| $R^2$ : P2PD versus time | 0.073 | 0.1 | 0.014 | 0.043 | 0.62 | 0.71 | 0.68 | 0.59 | 0.57 | 0.14 |

**Table 1.** Slope of P2PD over time and $R^2$ for Mouse # 1 and # 2 at oscillation frequencies of 100-1000 Hz.

## Discussion and conclusion

- In Mouse # 1, the P2PD ratio increased over time at each oscillation frequency.
- In Mouse # 2, the P2PD ratio stayed the same or increased depending on the oscillation frequency.
- Increase in P2PD ratio indicated that the tumor was stiffening over time relative to the background tissue.
- Tumor size increased over time. Method of calculating tumor size could be improved.
- Future work will analyze P2PD ratio in a larger sample of mice to explore why stiffness increased faster and slower in Mouse # 1 versus # 2 with histopathological validation.

## References

[1] Watkins EJ. JAAPA. 2019;32:13-17
[2] Bodelon C. et al. Breast Cancer Res. 2021;23:1-14
[3] Fenner J. et al. Sci Rep. 2014;4:1-8
[4] Sigrist RMS. et al. Theranostics. 2017;7:1-27
[5] Doherty JR. et al. IEEE Trans. Ultrason. Ferroelectr. Freq. Control. 2013;60:1-17
[6] Hossain MM. et al. IEEE Trans Med Imaging. 2021;40:1-15
[7] Hossain MM. et al. IEEE Trans Med Imaging. 2022

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